Evaluation of *Pseudomonas graminis* CPA-7 as a biopreservation method for fresh-cut pear. Physicochemical, enzymatic and nutritional quality

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Highlights

- Postharvest calcium treatment had a minimal effect on the quality of whole pear
- Postharvest calcium treatment and the addition of *Pseudomonas graminis* CPA-7 maintained the PPO activity of fresh-cut pears
- The firmness and activity values of PME and PG remained stable in fresh-cut pears under storage at 5 ± 1 °C

ABSTRACT

Biological preservation methods with bacterial antagonists have emerged as alternatives to chemical sanitizers for extending shelf-life and reducing the population of pathogenic microorganisms. In addition, calcium plays an important role in maintaining the quality of fruit, and postharvest calcium treatments might determine the potential of fruit for processing. The objective of this work was to evaluate the effect of the postharvest application of calcium and biopreservation with the CPA-7 of *P. graminis* on the quality parameters of fresh-cut pears. After harvest, whole pears were dipped in calcium chloride solution (1 %, w/v) or water (control) for 10 min at 25 °C and stored for 5 months at temperatures ranging from 0 to -0.5 °C. Both batches of fruit were minimally processed and dipped in a solution containing CPA-7 and an antioxidant solution or kept untreated, and both groups were stored at 4 °C for 6 d. The postharvest calcium treatment had no remarkable effect on the quality of the whole and fresh-cut pears. The enzymatic activities (PPO, PME and PG) related to browning and softening were constant in fresh-cut pears after storage, and the application of *P. graminis* CPA-7 had a positive effect on the activity of PPO. Finally, a combined effect of the biocontrol agent and calcium treatment was not demonstrated.

Keywords: Fresh-cut fruit and vegetables; antimicrobial agents; antioxidants; fruit postharvest; cold storage.

Abbreviations

AA: Antioxidant Activity

BI: Browning Index

DPPH$^{\cdot}$**:** 2,2-diphenyl-1-picrylhydrazyl

FRAP: Ferric Reducing Antioxidant Power

CPA-7: *Pseudomonas graminis* CPA-7

OLAF: oriented polyester

OPP: oriented polypropylene

PG: Polygalacturonase

PME: Pectin methyl esterase

POD: Peroxidase

PPO: Polyphenol oxidase

rPET: recycled polyethylene

SSC: Soluble Solids Content

TA: Titratable acidity

TPC: Total Phenolic Content

Introduction

Pears (*Pyrus communis* L.) have low protein, lipid and glucose contents and are rich in other sugars such as fructose, sorbitol, and sucrose. Pears also contain micronutrients, such as vitamins, minerals, and antioxidants (Colás-Medà *et al.*, 2015). The consumption of pears in Spain, which is currently 5.49 kg/year per person, decreased by 11.9 % in 2015 (MAGRAMA, 2016). In 2014, 373,900 tons of pears were produced in Spain, 50.4 % of which were produced in Lleida (MAPAMA, 2017).

In recent years, the society's lifestyle changes have caused an increase in the consumption of minimally processed fruits and vegetables. These products have desirable attributes such as freshness, low calorie contents, desirable nutritional composition and convenience (Colás-Medà *et al.*, 2015). The main problem with fresh-cut fruits and vegetables is their short shelf-life due to the damage caused during minimal processing by operations such as peeling, coring, slicing and shredding. These lead to an increase in respiration, water loss, biochemical changes and microbial spoilage (Ngamchuachit *et al.*, 2014; Plaza *et al.*, 2016). The acceptability of fresh-cut fruits and vegetables is determined by appearance and texture. Therefore, it is essential to understand which processes produce changes in the appearance and texture of these products to develop novel strategies for maintaining their quality (Toivonen and Brummell, 2008).

Surface treatments are necessary to delay physiological decay in fruit tissues, thus stabilizing the surface of the fruit and preventing degradative processes that limit the quality of the product (Soliva-Fortuny and Martín-Belloso, 2003). Currently, the main chemical compounds used for these treatments are calcium salts with calcium chloride being the most common. The application of calcium has been studied in postharvest for whole fruit (Manganaris *et al.*, 2007; Ghafir *et al.*, 2013; Sajid *et al.*, 2014; Kou *et al.*, 2015; Zhao and Wang, 2015) and fresh-cut fruit (Silveira, Aguayo and Artés, 2010; Ngamchuachit *et al.*, 2014), which is usually applied by dipping. The objective of the postharvest application of calcium to

whole fruit has been to reduce disorders such as brown spots in pears (Kou *et al.*, 2015) or bitter pits in apples (Sajid *et al.*, 2014). However, in fresh-cut fruit, calcium salts are usually used in combination with ascorbic acid or organic acids such as citric acid as antibrowning agents because they can inactivate enzymes such as polyphenol oxidase (PPO, EC 1.10.3.2) and peroxidase (POD, 11.1.1.x) and substrates from damaged cells (Soliva-Fortuny, Elez-Martínez and Martín-Belloso, 2004; Gomes *et al.*, 2010). Moreover, those solutions have been effective in maintaining the firmness of the fruit during storage due to its indirect effect on the activity of polygalacturonase (PG, EC 3.2.1.67) and pectin methyl esterase (PME, EC 3.1.1.11) (Gomes *et al.*, 2010; Ngamchuachit *et al.*, 2014). In addition, the use of calcium salts increases the content of calcium in fresh-cut fruit (Manganaris *et al.*, 2007).

Biological preservation has been presented as a promising alternative to chemical sanitizers to extend the shelf-life of fresh-cut fruit and to reduce the population of pathogenic microorganisms (Plaza *et al.*, 2016). Recently, the CPA-7 strain of *Pseudomonas graminis* has been isolated from apple and has been demonstrated to be effective for the reduction of the population of *Listeria monocytogenes* and *Salmonella* in fresh-cut apples, peaches and melon (Alegre, Viñas, Usall, Anguera, *et al.*, 2013; Alegre, Viñas, Usall, Teixidó, *et al.*, 2013; Abadias *et al.*, 2014) and in the maintenance of the nutritional quality and enzymatic activity in fresh-cut melon (Plaza *et al.*, 2016). Current studies using yeasts or bacterial antagonists are focused on pathogen control, and few of them focus on nutritional quality and enzymatic activity.

There have been few reports on the effect of postharvest calcium application to whole fruit and the effects of biological preservation on the quality, nutritional content and antioxidant properties of minimally processed fruit during refrigerated storage. The general objective of the present study was to investigate the effectiveness of postharvest calcium treatment and *Pseudomonas graminis* CPA-7 against the main postharvest fruit and vegetable pathogens and maintenance of the quality of whole and fresh-cut pears. We have divided this objective into two parts. The first (Iglesias *et al.*, 2018) addresses the effect of these treatments on foodborne pathogens and volatile compounds. In that paper was demonstrated that neither CPA-7 nor

112 $CaCl_2$ postharvest treatment were found to have an effect against *Salmonella enterica* under the
113 same conditions. However, CPA-7 significantly reduced (approximately 1-log unit) the
114 population of *Listeria monocytogenes*.

115 The second involves their effects on physicochemical, enzymatic and nutritional
116 qualities of the pears. Thus, the objective of second part was to evaluate the effect of postharvest
117 treatment with calcium and the antagonistic CPA-7 strain of *P. graminis* on the
118 physicochemical parameters, enzymatic activities (PPO, POD, PME and PG), vitamin C
119 content, total phenolic content, antioxidant activity and calcium content of minimally processed
120 pears throughout their shelf-life.

Material and methods

Plant materials and postharvest treatment

'Conference' pears were obtained from a local farmer (Grealó, Lleida) at commercial maturity (62.6 N; 12.6 % soluble solids; 2.46 g malic acid L^{-1}). Fruit free of visual defects and uniform in colour and size were selected and divided into two lots. The postharvest treatments consisted of dipping the fruit at 25 °C for 10 min in water (control) or 1 % $CaCl_2$. Then, the fruit were air dried at room temperature before storage at temperatures ranging from 0 to -0.5 °C for 5 months under controlled atmospheric conditions (2 % O_2 and 1 % CO_2).

Bacterial strain

The CPA-7 strain of *P. graminis* (deposit number CBS 136973, Centraalbureau voor Schimmelcultures, CBS, Utrech, The Netherlands), isolated in our lab from the surface of an apple (Alegre, Viñas, Usall, Teixidó, *et al.*, 2013), was used as antagonist. It was grown and inoculated as previously described (Iglesias *et al.*, 2018).

Processing, packaging and storage

As previously described by Iglesias *et al.* (2018), pears were cleaned, sanitized by immersion into a 0.1 g L^{-1} NaOCl solution adjusted to pH 6.5 using citric acid, rinsed, and dried prior to cutting. Pears were peeled and cut into 10 wedges using a handheld apple corer and slicer. Then, the cut pieces of fruit were dipped into an ice bath. The pears treated postharvest with calcium (1 % $CaCl_2$), and those left untreated were divided into 2 batches each. Each batch was dipped (1:2 w/v) for 2 min at 150 rpm (horizontal shaker) in cold water and in an antioxidant solution (2 % ascorbic acid, 2 % sodium citrate and 1 % calcium chloride). Two of these batches were treated with *P. graminis* CPA-7 (10^7 CFU mL^{-1}), and the results were compared to those of a negative control (without CPA-7 treatment). The treated fruit were air dried at room temperature. Approximately 110 g of cut pear samples was placed in recycled polyethylene (rPET) trays (120 x 120 x 55 mm, Plus Pack, Denmark) and heat-sealed with a

146 film consisting of biaxially oriented polyester (OLAF) anti-fog inner layer and an oriented
147 polypropylene (OPP) outer layer which contained lines of holes of 60-80 µm 75 mm apart from
148 each other. Afterwards, the pear trays were stored at 5 ± 1 °C for 6 d. At days 0, 2 and 6, a
149 portion of each sample was frozen with liquid nitrogen, pulverized and stored at -80 °C for
150 enzymatic assays, vitamin C analysis, phenolic content analysis, antioxidant activity and
151 calcium determination.

152 *Physicochemical assays*

153 Physical (colour and firmness) and chemical (titratable acidity and soluble solids
154 content) assays were performed according to methods described by Abadias et al. (2014) with
155 minor modifications as detailed by Plaza et al. (2016). Soluble solids contents were expressed as
156 % and titratable acidity as g of malic acid L^{-1}. Three determinations were performed per
157 treatment and sampling time. Firmness evaluation was carried out by determination of the
158 maximal strength necessary for a cylindrical probe 4 mm in diameter and plane basis to
159 penetrate 10 mm into a pear sample using a TA-TX2 Texture Analyser (Stable Micro Systems
160 Ltd., Surrey, England). Briefly, colour was measured with a CR-200 Minolta Chroma Meter
161 (Minolta, INC., Tokyo, Japan) with illuminant D65 and a 10° observer angle. . L* defines the
162 lightness, and a* and b* define the red-greenness and blue-yellowness, respectively. These
163 values were used to calculate the browning index (BI) described by Liu et al. (2016).

164 *Assessment of overall visual quality*

165 The assessment was carried out as described by Altisent et al. (2014). The visual quality
166 was determined by an untrained panel based on the following hedonic scale: 9 = excellent; 7 =
167 very good; 5 = good, limit of marketability; 3 = fair, limit of usability; and 1 = poor, inedible.

168

169 *PPO and POD activity*

170 The PPO and POD activities were measured as described by Plaza et al. (2016). A PPO
171 unit was defined as 0.01 absorbance units per minute, and PPO activity was defined as the
172 change in absorbance per second per kilogram on a fresh weight basis. A POD unit was defined
173 as 0.01 absorbance units per minute, and POD activity was defined as the change in absorbance
174 per second per kilogram on a fresh weight basis.

175 *PME and PG activity*

176 PME activity was determined according to the method of Plaza et al. (2003) with some
177 modifications. Briefly, the enzyme was extracted by the homogenization of 5 g of frozen sample
178 with 10 mL of an extraction solution (1 mol L^{-1} NaCl in 0.2 mol L^{-1} sodium phosphate buffer,
179 pH 7.5). The resulting mixture was shaken for 10 min at 4 °C, centrifuged at 13523 × g for 20
180 min at 4 °C and then filtered. The supernatant was then collected and stored at -80 °C until the
181 determination of enzyme activity. PME activity was measured titrimetrically at 25 °C. Briefly, 2
182 mL of the enzymatic extract were mixed with 40 mL of a pectin-salt substrate solution (0.35 %
183 pectin and 0.1 mol L^{-1} NaCl). The solution was adjusted to pH 7.5 with 1 mol L^{-1} NaOH. After
184 the pH reached 7.5, 0.1 mL of 0.05 mol L^{-1} sodium hydroxide was added. The time elapsed until
185 the pH of the solution regained pH 7.5 was measured. A PME unit was defined on a fresh
186 weight basis as the amount of enzyme required to release 1 mol of carboxyl groups per second.

187 PG activity was determined according to the method of Van linden et al. (2008) with
188 some modifications. Six grams of frozen sample were homogenized with 9 mL of cold
189 deionized water. The mixture was adjusted to pH 3.0 using HCl, stirred for 5 min and
190 centrifuged at 13523 x g for 20 min at 4 °C. The pellet was recovered and resuspended in 6 mL
191 of cold 1.2 mol L^{-1} NaCl, the solution was adjusted to pH 6.0 and stirred for 5 min and then
192 centrifuged at 13523 x g for 20 min at 4 °C. Finally, the collected extract was stored at -80 °C
193 until PG determination. Reaction mixtures consisted of 350 µL of a 0.2 % (w/v) buffered PGA
194 solution containing 0.04 mol L^{-1} Na acetate at pH 4.4 and 50 µL of PG extract. The reaction

mixtures were then incubated at 40 °C for 10 min. Two millilitres of cold borate buffer (pH 9.0, 0.1 mol L^{-1}) were added to each sample to stop the reaction, and 0.4 mL of a 1 % 2-cyano-acetamide solution were added. The mixture was incubated for 10 min at 100 °C and then immediately cooled in an ice bath. The absorbance was determined using a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific™, Spain) at 276 nm and 22 °C. A standard calibration curve was generated at 276 nm using known concentrations of galacturonic acid (ranging from 0 to 3 x 10^{-3} mol L^{-1} final volume). The enzyme activity was calculated on a fresh weight basis as the release of reducing groups per second.

Vitamin C analysis

The assay was carried out as described by Altisent et al. (2014). The reduction of dehydroascorbic acid to ascorbic acid using TCEP as the reducing agent was used to determine the total vitamin C content. Total vitamin C was determined using high-performance liquid chromatography (HPLC). The results were expressed on a fresh weight basis as g of ascorbic acid per kg.

Determination of the total phenolic content

The total phenolic content was determined by the Folin-Ciocalteu method (Singleton, Orthofer and Lamuela-Raventós, 1999) employing the modifications described by Altisent et al. (2014). The results were expressed on a fresh weight basis as g of gallic acid equivalent per kg.

Determination of antioxidant activity

The antioxidant activity was determined by two different methods, namely, the 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radical scavenging and ferric reducing antioxidant power (FRAP) assays. The extraction and assays were carried out according to the methods described by Plaza et al. (2016). The results were expressed on a fresh weight basis as moles of ascorbic acid equivalents per kg.

220 *Determination of calcium*

221 The determination of calcium was performed using a HORIBA Jobin Yvon ICP
222 Spectrometer Model Activa inductively coupled plasma optical emission spectrometry (ICP-
223 OES) equipped with a Meinhard nebulizer. The instrument was calibrated using a single-
224 element atomic absorption standard solution of calcium (1000 mg L^{-1}). The sample pre-
225 treatment (digestion procedure) was carried out using an MLS 1200 mega microwave
226 (Milestone, Milan, Italy) with HNO_3 (700 mL L^{-1}), H_2O_2 (300 mL L^{-1}) and nanopure water. The
227 optical emission was measured at 393 nm. The results were expressed on a fresh weight basis as
228 g of calcium per kg.

229 *Statistical analysis*

230 The results were expressed as the means ± standard deviation. Differences among
231 treatments were evaluated by using one-way analysis of variance (ANOVA) and were
232 significant at $P<0.05$ (95 % confidence level). Tukey's test and *t*-tests methods were used to
233 determine differences between means. JMP 8 software was used to conduct ANOVA statistical
234 analyses (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Physical and chemical parameters and overall visual quality

The results of the physical and physicochemical parameters of whole and fresh-cut pears are shown in Table 1 and Table 2, respectively. The postharvest application of 1 % $CaCl_2$ to whole pear did not affect the colour or soluble solids content (SSC) after a storage period of 5 months. However, the results showed that calcium treated whole pears presented higher acidity and lower firmness than non-treated samples probably due to long-storage time (5 months). Moreover, Trentham et al. (2008) suggested that the concentration of calcium needed in the tissues to exert an effect on the firmness of the fruit should be 0.8-1.0 g kg^{-1}. Sajid et al. (2014) observed that pears cv. Le-Conte treated with 3 % $CaCl_2$ for 5, 10 or 15 min did not present SSC values that were significantly different from those of a control. However, Zhao and Wang (2015) reported that apples (*Malus domestica* Borkh. cv. Shouhong) dipped in 5 % $CaCl_2$ for 10 min presented higher SSC and firmness values than the corresponding values of untreated apples. Similar results were obtained by Ghafir et al. (2013) who reported that 'Red Delicious' (*Malus domestica*) apples treated with 1 % $CaCl_2$ presented higher firmness and SSC values and lower total acidity than untreated apples.

Overall, neither calcium chloride nor *Pseudomonas graminis* CPA-7 affected the firmness of fresh-cut pears (Table 2). Moreover, there were no significant differences at any point in the storage period for either treatment. However, fresh-cut pears subjected to only a postharvest calcium treatment presented higher BI (26.01 ± 15.09) compared to un-treated samples (17.78 ± 5.85) after a 6-day storage period at 5 ± 1 °C. In samples treated with *P. graminis* CPA-7, postharvest treatment with calcium had no effect in their BI after a 6-day storage period at 5 ± 1 °C. In addition, the BI of fresh-cut pears increased during storage at 5 ± 1 °C in the case of all treatments. Oms-Oliu et al. (2010) suggested that formulations composed of calcium and ascorbic were not completely effective in controlling the enzymatic browning of

fresh-cut fruits, since once the ascorbic acid is completely oxidized to dehydroascorbic acid, the *o*-quinones were no longer reduced and browning might appear.

The results of visual quality analysis obtained for fresh-cut samples were indirectly related to their BI (Fig. 1). As the BI value increased, the visual quality score of the sample decreased. After 6 d of storage at 5 ± 1 °C, the highest visual value (5.2 ± 1.0) was obtained for fresh-cut pears that had not been treated with either calcium or CPA-7. This was the only sample that was still marketable after six days of storage, and that limited their shelf-life (6 d).

Enzymatic activity

Polyphenol oxidase (PPO) and peroxidase (POD) assays

The postharvest application of 1 % $CaCl_2$ had no effect on the PPO and POD activities in whole pear after 5 months of storage under CA (Table 1). Overall, in fresh-cut pears, the PPO activity decreased or remained constant during the 6-day storage period at 5 ± 1 °C regardless of the applied treatment (Table 2). In calcium-untreated fresh-cut pears, the presence of CPA-7 could reduce the PPO activity during the 6-d storage period. However, Collazo et al. (2018) reported that PPO activity of fresh-cut apples increased drastically in response to CPA-7 in MAP reaching almost 8-fold the activity showed by the non-inoculated control stored in the same conditions. Moreover, Plaza et al. (2016) showed that after 8 d of refrigerated storage at 5 °C, fresh-cut melons inoculated with CPA-7 and packaged under MAP did not present significant changes with regard initial values. The inactivation of PPO could be associated with the antioxidant solution used in this study, which contained 2 % ascorbic acid (Altunkaya and Gökmen, 2008) more than the action of the antagonistic microorganism. Indeed, Arias et al. (2007) demonstrated that ascorbic acid did not inactivate pear-derived PPO, but it did decrease the activity of PPO by reacting with the product of the PPO-catalysed reaction.

Overall, the POD activities of both treated and untreated samples increased during the 6 d of storage at 5 ± 1 °C (Table 2). The same trend was observed in other studies with different food matrices and conservation treatments. For example, Xu et al. (2015) studied the effect of

high-pressure nitrogen treatments on the activity of POD in fresh-cut pear (*Pyrus nivalis*), and the results suggested that the activity increased during 14 d of storage at 4 °C. Plaza et al. (2016) reported that the POD activities in untreated fresh-cut melon (*C. melo* L.) and those treated with *P. graminis* CPA-7 increased after 8 d of storage at 5 °C. Moreover, Liu et al. (2016) observed that the POD activity increased during storage in fresh-cut apples treated with ozone. Rojas-Graü et al. (2008) did not observe any influence from the 1 % ascorbic acid antibrowning solution on the POD activity of fresh-cut 'Fuji' apple (*Malus domestica* Borkh.). The POD activity was significantly lower after storage in samples treated with *P. graminis* CPA-7 regardless of the calcium treatment. In the recent study carried out by Collazo et al. (2018), POD activity was inhibited immediately after *P. graminis* CPA-7 inoculation in fresh-cut 'Golden delicious' apples. However, in that study after 6 d of storage time in MAP, POD activity increased in the *P. graminis* CPA-7 inoculated samples (2.6-folder higher) respect to control.

The increase in the BI of fresh-cut pears observed in the present study during storage at 4 °C could be related to the increase in the POD activity found during the same period. Browning in fresh-cut fruits and vegetables has been associated with the PPO activity; however, several studies suggested that POD could also play a key role (Toivonen and Brummell, 2008). The increase observed in BI could also be due to secondary browning, which is usually associated with the germination of fungal spores on the cut surface (Toivonen, 2006).

Pectin methyl esterase (PME) and Polygalacturonase (PG) assays

Postharvest treatment with $CaCl_2$ produced a significant decrease ($P<0.05$) in the PME activity of whole pear, but no effect was observed in the PG activity (Table 1). However, some authors suggested that PME activity should increase in the presence of calcium salts (Alandes *et al.*, 2006, 2009; Quiles *et al.*, 2007). The results contradict those obtained by Manganaris et al. (2007) who reported that the PME activities of peach samples (*Prunus persica*, cv. 'Andross') dipped in calcium chloride, lactate, or propionate were higher than those of the control. Ortiz et

al. (2011) suggested that postharvest dips in 2 % $CaCl_2$ for 5 min did not enhance the PME activity in 'Golden Reinders' apples (*Malus x domestica* Borkh., cv. Golden Reinders) perhaps due to less intense effects from ethylene production. Moreover, they suggested that the PME activity was not directly related to the softening of the fruit. Therefore, the reduction in the firmness values obtained for whole pear (Table 1) might be more related to the PG activity than the PME activity. However, the demethylation of pectin (polymer of galacturonic acid) by PME is necessary for the depolymerization by PG in pear softening (Toivonen and Brummell, 2008; Ortiz, Graell and Lara, 2011).

In fresh-cut pears (Table 2), the lowest PME activities (0.84 ± 0.14 and 1.14 ± 0.03 x 10^{-5} mol s^{-1} kg^{-1}) were observed in pears subjected to postharvest calcium treatments at sampling day 0. Although the PG activity was higher in fresh-cut pears treated with calcium and *P. graminis* CPA-7 (1.91 ± 0.42 x 10^{-5} mol s^{-1} kg^{-1}) than in untreated samples (0.93 ± 0.21 x 10^{-5} mol s^{-1} kg^{-1}), no significant differences were observed. The maintenance of the PME and PG activities in all fresh-cut samples throughout the storage period could be related to the preservation of their firmness values. The dipping treatment used in the fresh-cut experiment consisting of ascorbic acid, sodium citrate and calcium chloride might have had a strong effect on these results by rinsing enzymes and substrates away from injured cells at the cut surfaces (Soliva-Fortuny and Martín-Belloso, 2003; Toivonen and Brummell, 2008).

Vitamin C

The vitamin C content was not affected by postharvest calcium treatment in whole pear (Table 1). In fresh-cut pears (Table 3), the initial vitamin C contents were very high due to the dipping treatment with the antioxidant solution containing ascorbic acid. However, the vitamin C concentration dramatically decreased from day 0 to 2, and then it remained constant for the remainder of the storage period. Ascorbic acid is typically located in the vacuole of the cell and is protected from oxidation by low pH and phenolic flavonoids (Cocci *et al.*, 2006; Hajilou and Fakhimrezaei, 2013). However, exogenous ascorbic acid is probably housed in a different

location and is therefore more exposed to O_2 (Cocci *et al.*, 2006), enzyme ascorbate oxidase or oxidizing enzymes such as POD (Martíns *et al.*, 2016). Fresh-cut pears treated with antagonist *P. graminis* CPA-7 presented the lowest vitamin C contents (0.12 ± 0.01 and 0.13 ± 0.01 g kg^{-1}) after 6 d of storage at 5 ± 1 °C. However, Collazo et al. (2018) recently reported that vitamin C contents remained constant along storage time (6 d, 5 °C) and there were no differences between *P. graminis* CPA-7 inoculated and non-inoculated fresh-cut apples at any of the analysed sampling points (0, 1, 3 and 6 d). Moreover, in the study carried out by Plaza et al. (2016), the application of *P. graminis* CPA-7 to fresh-cut melon had no effect on the vitamin C content. Therefore, *P. graminis* CPA-7 might use the ascorbic acid in pears as a nutrient.

The total phenolic content and antioxidant activity

Postharvest calcium treatment had no effect on the total phenolic content of whole pears (Table 1). Similar results were obtained by Zhao and Wang (2015) who observed that the phenolic content of apples (*Malus domestica* Borkh. cv. Shouhong) treated with 5 % $CaCl_2$ for 10 min and stored for 28 d were not different from those of the control. However, Kou et al. (2015) reported that 'Huangguan' pears (*P. pyrifolia* Nakai cv. Huangguan) dipped in 2 % $CaCl_2$ for 15 min and stored for 8 months showed a higher contents of individual phenolic compounds than untreated pears. Overall, the total phenolic contents of fresh-cut pears decreased ($P>0.05$), and there were no significant differences between treatments after 6 d of storage at 5 ± 1 °C (Table 3). This might be due to progressive oxidation of the antioxidant solution (calcium chloride, ascorbic acid and sodium citrate). The cyclic structures of ascorbic acid and citric acid are very similar to the structure of phenolic compounds, and they could interfere in the spectrophotometric determination of the total phenolic content (Singleton, Orthofer and Lamuela-Raventós, 1999; Siddiq, Sogi and Dolan, 2013).

For whole pears treated with calcium, after 5 months storage, the antioxidant activity decreased in the $DPPH^{\cdot}$ assay and increased in the FRAP assay (Table 1). The differences observed could be attributed to the different principles of each assay. Both assays are redox-linked colorimetric methods, but the $DPPH^{\cdot}$ assay is based on accepting hydrogen (H) atoms

(Mishra, Ojha and Chaudhury, 2012), and the FRAP assay is indicative of taking electrons from antioxidants (Benzie and Strain, 1996). The antioxidant activity decreased in fresh-cut pears during 6 d of storage at 5 ± 1 °C (Table 3). Moreover, fresh-cut pears subjected to a postharvest calcium treatment presented the highest antioxidant activities for both assays ($P<0.05$). Fresh-cut pears treated with *P. graminis* CPA-7 presented the lowest antioxidant potentials when measured using the FRAP method ($1.52 \pm 0.01 \times 10^{-3}$ and $1.89 \pm 0.02 \times 10^{-3}$ mol kg^{-1}) for those fresh-cut pears with or without postharvest calcium treatment, which is consistent with the vitamin C contents. However, in a previous study carried out by Plaza et al. (2016), no significant differences ($P>0.05$) were observed between fresh-cut melons (*C. melo* L.) treated with *P. graminis* CPA-7 and the control after 8 d of storage at 5 °C. The results suggested that *P. graminis* CPA-7 could use antioxidant compounds such as ascorbic acid as nutrients. Further studies are needed to confirm this and to study the mode of action of this microorganism, including the competition for nutrients in different food matrices.

Calcium content

The calcium contents in whole pears are listed in Table 1. There were no significant differences ($P>0.05$) between untreated and treated whole pears. The results contradict those obtained by Kou et al. (2015) who reported that the application of 2 % $CaCl_2$ to 'Huangguan' pears (*P. pyrifolia* Nakai cv. Huangguan) increased the concentration of calcium. Moreover, Manganaris et al. (2007) observed that the application of calcium salts increased the content of calcium in the peel and flesh of peach (*Prunus persica*, cv. 'Andross'). Chardonnet et al. (2003) suggested that 2 % calcium chloride dip was enough to achieve maximum calcium accumulation in the cell wall of apples. 'Conference' pears might have reached saturated levels of calcium in their cell walls during formation and ripening and the addition of external calcium had no effect in the total content of calcium.

The calcium content in fresh-cut pears was higher than in whole pears (≈ 2 g kg^{-1}) due to the applied antioxidant treatment that contained 1 % $CaCl_2$. The content of calcium was

391 maintained in fresh-cut pears after 6 d of storage at 5 ± 1 °C, except for fresh-cut pears not
392 subjected to postharvest calcium treatment and treated with *P. graminis* CPA-7 after cutting
393 (Table 3). To the best of our knowledge, in the studies carried out with *P. graminis* and
394 specifically with *P. graminis* CPA-7 (Alegre, Viñas, Usall, Anguera, *et al.*, 2013; Alegre, Viñas,
395 Usall, Teixidó, *et al.*, 2013; Abadias *et al.*, 2014; Oliveira *et al.*, 2015; Plaza *et al.*, 2016), there
396 is no mention of the use of calcium as a nutrient. The lowest content of calcium was observed in
397 fresh-cut pears subjected to a postharvest calcium treatment (2.05 ± 0.00 and 2.02 ± 0.01 g kg^{-1})
398 after 6 d of storage at 5 ± 1 °C. The postharvest calcium treatment had no influence on the
399 calcium content of fresh-cut pears. The flesh and peel of pears, where calcium is normally most
400 concentrated, were eliminated during processing, and thus the postharvest calcium treatment
401 might have had no effect on the final calcium content in fresh-cut pears. However, a significant
402 effect on the final calcium content was caused by the antioxidant solution, which contained
403 calcium chloride.

Conclusions

The effects of postharvest calcium treatment and bioconservation using *Pseudomonas graminis* CPA-7 on the enzymatic activities and nutritional qualities of fresh-cut pears were evaluated. Postharvest calcium application had a minimal effect on the quality of whole 'Conference' pears. Fresh-cut pears could be the most suitable option for increasing the consumption of pears in Spain and throughout Europe. Consumers usually demand fresh-cut fruits or vegetables with no symptoms of browning or softening. In general, firmness values and PME, PG and PPO activity values were maintained in fresh-cut pears after 6 d of storage at 5 ± 1 °C. It was shown that the application of *P. graminis* CPA-7 had a positive effect on maintaining the PPO activity, which is usually associated with the browning index and limited the shelf-life of fresh-cut products. In this study, a clear synergy between calcium chloride and *P. graminis* CPA-7 in fresh-cut pears was not observed. Future studies should include the application of *P. graminis* CPA-7 in extreme conditions (for example without a disinfection step) and the use of ascorbic acid and phenolic compounds as nutrients by bacteria. Moreover, descriptive tests involving a trained panel and emerging sensory methods with consumers are necessary.

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563 10.1007/s10068-015-0145-5.

564

565 **FIGURE CAPTIONS**

566

567 **Fig. 1** Effect of postharvest calcium chloride treatment and biopreservation using *Pseudomonas*
568 *graminis* CPA-7 [NoCa/NoCPA7 (♦), NoCa/CPA7 (▲), Ca/NoCPA7 (■) and Ca/CPA7 (×)] on
569 the overall visual quality of fresh-cut pears during 6 d of storage at 5 ± 1 °C. The error bars
570 represent the standard deviation with a 95 % of confidence interval**.**

571

572 **TABLES**

573 **Table 1** Quality parameters of whole pear after 5 months of storage at -0.5 °C. Values are the
574 means of independent determinations ± standard deviation. Different letters in the same row
575 indicate significant differences (P<0.05). [a] OD, optical densitometry

Parameters	No Calcium	Calcium
Acidity (g malic acid L^{-1})	1.52 ± 0.04 b	1.79 ± 0.05 a
Soluble solid content (%)	14.17 ± 0.06 a	14.20 ± 0.00 a
Firmness (N)	16.79 ± 0.81 a	14.45 ± 1.28 b
*L**	75.52 ± 2.53 a	76.34 ± 3.65 a
*a**	-1.87 ± 0.39 a	-1.85 ± 0.51 a
*b**	12.62 ± 1.56 a	14.08 ± 2.40 a
PPO (ΔOD s^{-1} kg^{-1})[a]	469.49 ± 90.71 a	587.93 ± 24.99 a
POD (ΔOD s^{-1} kg^{-1})	433.84 ± 44.60 a	629.19 ± 126.47 a
PME (mol s^{-1} kg^{-1}) x 10^{-5}	0.97 ± 0.03 a	0.72 ± 0.06 b
PG (mol s^{-1} kg^{-1}) x 10^{-5}	0.80 ± 0.08 a	1.25 ± 0.28 a
Vitamin C (g kg^{-1})	0.02 ± 0.00 a	0.02 ± 0.00 a
Phenols (g kg^{-1})	0.26 ± 0.03 a	0.23 ± 0.02 a
Antioxidant activity		
$DPPH^{\cdot}$ (mol kg^{-1}) x 10^{-3}	1.20 ± 0.07 a	0.91 ± 0.10 b
FRAP (mol kg^{-1}) x 10^{-3}	0.69 ± 0.04 b	0.81 ± 0.04 a
Calcium (g kg^{-1})	0.49 ± 0.01 a	0.50 ± 0.00 a

576

577

578 **Table 2** Physical parameters and enzymatic activities of fresh-cut pears during storage at 5 ± 1 °C. Values are the means of independent determinations ±
579 standard deviation. Different capital letters in the same treatment indicate significant differences between days (P<0.05). Different lower-case letters in the
580 same storage day indicate significant differences (P<0.05) between treatments. [a] OD, optical density

Treatments	Storage time (days)	Firmness (N)	Browning Index	PPO (ΔOD s^{-1} kg^{-1}) [a]	POD (ΔOD s^{-1} kg^{-1})	PME (mol s^{-1} kg^{-1}) x 10^{-5}	PG (mol s^{-1} kg^{-1}) x 10^{-5}
NO CALCIUM							
Control	0	14.03 ± 3.70 Aa	12.90 ± 3.42 Ba	390.01 ± 44.50 Ab	329.76 ± 11.70 Cab	1.21 ± 0.16 Aab	1.21 ± 0.32 Aa
	2	11.98 ± 2.15 Ab	15.68 ± 5.04 ABb	424.81 ± 39.31 Aa	569.65 ± 3.69 Ba	1.33 ± 0.28 Aa	1.01 ± 0.04 Ab
	6	14.09 ± 1.51 Aa	17.78 ± 5.85 Ab	359.59 ± 8.83 Aa	673.89 ± 36.42 Aa	1.28 ± 0.07 Aa	0.93 ± 0.21 Ab
CPA-7							
	0	11.61 ± 1.15 Aa	13.96 ± 3.31 Ba	523.06 ± 9.35 Aa	389.69 ± 54.65 Bab	1.58 ± 0.20 Aa	0.88 ± 0.14 Ba
	2	13.33 ± 3.74 Aab	18.07 ± 7.85 ABb	396.89 ± 19.85 Ba	557.21 ± 28.80 Aa	1.30 ± 0.11 Aa	2.25 ± 0.48 Aa
	6	12.54 ± 3.15 Aa	20.55 ± 7.00 Aab	252.88 ± 13.78 Cc	566.79 ± 64.38 Ab	1.34 ± 0.15 Aa	1.35 ± 0.14 Bab
CALCIUM							
Control	0	13.20 ± 2.27 Aa	15.50 ± 2.60 Ba	515.13 ± 16.41 Aa	445.24 ± 12.31 Ca	0.84 ± 0.14 Ab	1.35 ± 0.10 Aa
	2	16.14 ± 2.64 Aa	23.39 ± 11.39 ABa	372.11 ± 58.55 Ba	527.29 ± 33.02 Ba	1.29 ± 0.29 Aa	1.58 ± 0.23 Aab
	6	13.56 ± 1.88 Aa	26.01 ± 15.09 Aa	305.49 ± 14.44 Bb	604.18 ± 19.64 Aa	1.13 ± 0.14 Aa	1.53 ± 0.22 Aab
CPA-7	0	10.46 ± 2.07 Aa	13.63 ± 2.40 Ba	345.50 ± 36.34 Ab	321.49 ± 47.76 Bb	1.14 ± 0.03 Ab	1.41 ± 0.42 Aa
	2	15.42 ± 2.94 Aab	19.59 ± 5.01 Aab	391.74 ± 50.21 Aa	515.65 ± 33.49 Aa	1.38 ± 0.26 Aa	1.74 ± 0.27 Aab
	6	13.29 ± 2.07 Aa	19.29 ± 4.22 Ab	309.04 ± 7.62 Ab	556.15 ± 16.59 Ab	1.00 ± 0.25 Aa	1.91 ± 0.42 Aa

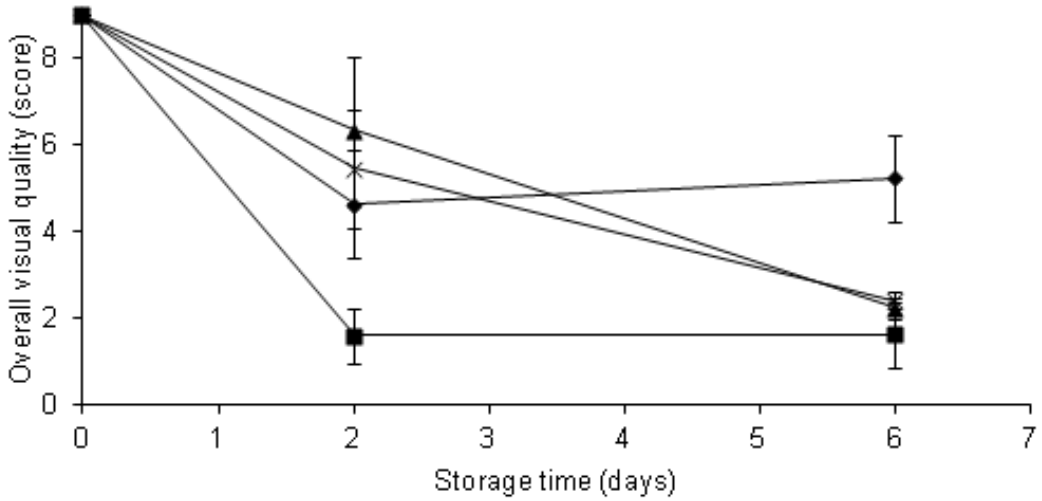
581

582 **Table 3** Vitamin C, phenol, and calcium contents and antioxidant activity of fresh-cut pears during storage at 5 ± 1 °C. Values are the means of independent
583 determinations ± standard deviation. Different capital letters in the same treatment indicate significant differences between days (P<0.05). Different lower-
584 case letters in the same storage day indicate significant differences (P<0.05) between treatments

Treatments	Storage time (days)	Vitamin C (g kg^{-1})	Phenols (g kg^{-1})	$DPPH^{\cdot}$ (mol kg^{-1}) x 10^{-3}	FRAP (mol kg^{-1}) x 10^{-3}	Calcium (g kg^{-1})
NO CALCIUM						
Control	0	1.53 ± 0.10 Aa	0.79 ± 0.11 Aa	5.34 ± 0.09 Aa	5.06 ± 0.16 Aa	2.14 ± 0.02 Bb
	2	0.18 ± 0.04 Bbc	0.61 ± 0.03 ABa	1.11 ± 0.16 Bb	2.27 ± 0.01 Ba	2.34 ± 0.03 Aa
	6	0.16 ± 0.01 Ba	0.57 ± 0.07 Ba	1.35 ± 0.02 Bb	1.85 ± 0.03 Cb	2.08 ± 0.00 Bab
CPA-7						
	0	1.26 ± 0.05 Aab	0.74 ± 0.05 Aa	3.93 ± 0.18 Ab	4.23 ± 0.32 Ab	2.37 ± 0.01 Aa
	2	0.22 ± 0.00 Bab	0.47 ± 0.03 Bb	1.58 ± 0.14 Ba	1.76 ± 0.10 Bb	2.02 ± 0.02 Cc
	6	0.12 ± 0.01 Cc	0.54 ± 0.05 Ba	1.04 ± 0.12 Cb	1.52 ± 0.01 Bc	2.09 ± 0.01 Ba
CALCIUM						
Control	0	1.30 ± 0.17 Aab	0.94 ± 0.10 Aa	5.18 ± 0.06 Aa	5.07 ± 0.44 Aa	2.08 ± 0.03 Bb
	2	0.24 ± 0.00 Ba	0.56 ± 0.04 Bab	1.35 ± 0.14 Bab	1.98 ± 0.04 Bb	2.18 ± 0.01 Ab
	6	0.15 ± 0.01 Bab	0.56 ± 0.06 Ba	1.51 ± 0.09 Bab	2.00 ± 0.07 Ba	2.05 ± 0.00 Bbc
CPA-7	0	1.04 ± 0.13 Ab	0.75 ± 0.08 Aa	3.91 ± 0.24 Ab	4.14 ± 0.12 Ab	2.10 ± 0.08 Ab
	2	0.16 ± 0.01 Bc	0.50 ± 0.05 Bb	1.58 ± 0.12 Ba	1.94 ± 0.07 Bb	2.00 ± 0.03 Ac
	6	0.13 ± 0.01 Bbc	0.60 ± 0.06 ABa	1.99 ± 0.38 Ba	1.89 ± 0.02 Bb	2.02 ± 0.01 Ac

585
586

587 **Figure 1**



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